Issued November 27, 1909.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY—BULLETIN 121.

A. D. MELVIN, CHIEF OF BUREAU.

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BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., November 2, 1909.

Sir: I have the honor to transmit herewith and to recommend for publication in the bulletin series of this Bureau a manuscript entitled "The Need of Controlling and Standardizing the Manufacture of Veterinary Tetanus Antitoxin," by Dr. John R. Mohler, chief of the Pathological Division of this Bureau, and Dr. Adolph Eichhorn, bacteriologist in that division.

The investigation reported in this paper was undertaken in accordance with the act of Congress making appropriations for the Department of Agriculture for the past fiscal year, which empowered the Secretary of Agriculture to purchase in the open market samples of tuberculin, serums, antitoxins, etc., designed for veterinary use, to test the same, and to publish the results of such tests. This act does not, however, confer any authority to supervise or control the manufacture of these products.

It has become known in the veterinary profession that tetanus antitoxin for veterinary use—unlike that for human use, which is manufactured under stringent government supervision—is urgently in need of standardization. Under existing conditions the veterinarian and the stock owner are at the mercy of the manufacturers of this antitoxin, some of whom make no statement as to the strength of their product, while in the great majority of the cases tested in the present investigation it was found that the standard was not reached and many of the samples were far below it.

It is very necessary that the veterinarian should have some reliable assurance of the strength of this most valuable therapeutic agent. There is, therefore, pressing need for legislation empowering the Secretary of Agriculture to supervise and control the manufacture of veterinary tetanus antitoxins and to prescribe and enforce a proper standard of potency. Such authority, of course, would be limited to products manufactured for interstate commerce or offered for importation.

Very respectfully. A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE NEED OF CONTROLLING AND STANDARDIZING THE MANUFACTURE OF VETERINARY TETANUS ANTITOXIN.

INTRODUCTION.

Of the various biological products prepared for the cure and prevention of infective diseases in animals tetanus antitoxin has been probably the most extensively used by the veterinary profession. The good effects which were expected from the administration of this antitoxin in the prophylactic and curative treatment of tetanus have not, however, been all that could be desired, and it is surprising to learn of the divergent results which are obtained from its use by various practitioners. It is therefore only natural that tetanus antitoxin has not gained the favor of the veterinarian and is not used to the extent merited by such a valuable product. It has been suggested that the lack of uniform results which has followed the administration of the antitoxin is primarily due to the variation in the strength of the product, and following numerous requests from different sources it was decided to undertake the standardization of veterinary tetanus antitoxins prepared by different manufacturers, and also to determine whether these products are subject to any variations in strength. This work seemed particularly desirable in view of the address of Anderson before the June meeting of the American Medical Association, in which he stated that his experiments showed veterinary tetanus antitoxin to contain as low as 17 to 25 antitoxin units per cubic centimeter, while similar examinations of antitoxin prepared for medical use contained from 150 units to 600 or even 700 units per cubic centimeter.

The investigation herein recorded was carried out in accordance with an act of Congress making appropriations for the Department of Agriculture for the year ending June 30, 1909, which provides as follows:

> That the Secretary of Agriculture is authorized to purchase in the open market samples of all tuberculin, serums, antitoxins, or analogous products, of foreign or domestic manufacture, which are sold in the United States for the detection, prevention, treatment, or cure of diseases of domestic animals, to test the same, and to publish the results of said tests in such a manner as he may deem best.

It should be noticed that while power has been granted to purchase, test, and publish upon these products, no authority has been given with reference to the supervision and control of their manufacture. This paper is presented with the object of furnishing some concrete examples of the variation observed in tetanus antitoxin at present on the market, and to show the necessity for Federal supervision of all vaccines, serums, antitoxins, viruses, and analogous products, including mallein, tuberculin, anthrax and blackleg vaccine, and hog-cholera serum.

NATURE AND CAUSE OF TETANUS.

Tetanus is an extremely painful disease of all animals, including man, but it more particularly affects the horse, ass, and mule. It is caused by the *Bacillus tetani*. This organism differs from other disease-producing bacteria in the fact that it remains localized at the point of entrance to the body, which is usually through a wound. Here it multiplies and produces a powerful toxin which is absorbed into the circulation and, by acting on the nervous system, gives rise to the symptoms of the disease. From this it will be seen that tetanus is the type of the purely toxic disease and is not transmissible by contact unless the wound containing the germ is discharging pus or blood, as may be the case in tetanus following the operation of castration. Neither can the disease be transmitted from one animal to another by blood inoculation, because no bacilli are circulating in the blood. The bacillus is a spore former, and these spores are very resistant to antiseptics. It can live outside the animal body, and is quite prevalent in garden earth and in the neighborhood of manure piles. It is an anaerobic organism like the bacillus of blackleg, and consequently can multiply only in the absence of oxygen. Deep punctured wounds about the feet and legs are especially serious, as in wounds of this kind the bacilli are most likely to be implanted, and once they have gained access the absence of oxygen in these deep wounds makes the conditions favorable for their growth. The period of incubation varies from a few days to two weeks.

The symptoms come on gradually. There is stiffness of the gait and rigidity of the muscles, the appearance of the animal somewhat resembling a horse foundered in all four feet. The neck is rigid, the head extended, and the tail elevated and immovable. The characteristic symptom of tetanus is the protrusion of the nictitans membrane over the eye when the head is raised. This condition is due to a contraction of the muscles of the eyeball drawing it back into its socket and forcing out the nictitans membrane. There is no other disease in which this condition is present. The animal is extremely nervous, starts at the slightest sound, loses its appetite, and sweats

freely. There is a spasmodic contraction of certain groups of muscles, especially those of the jaw, so that the animal is unable to open its mouth, from which the disease derives its popular name of lockjaw. The malady runs an irregular course and death may occur in a few days or the affection may be prolonged over two or three weeks. Spontaneous recovery is very doubtful, and the outlook is always grave. The mortality for some classes of animals has been given as almost 100 per cent, while about 75 per cent of the cases in horses terminate fatally. Unfortunately the disease is seldom suspected until a relatively large amount of toxin has formed and begun to manifest its action in the patient's body.

HISTORICAL SUMMARY.

Prior to the discovery of the cause of tetanus there were various theories advanced regarding the character of the disease. One of the most widely accepted views was that tetanus, since it generally develops as a result of an injury, was occasioned by the tearing or contusion of some of the peripheral nerves and as a consequence the changes produced were conveyed to the spinal cord. On the other hand, in the cases where no injury was associated with the disease it was thought to be of idiopathic origin.

With the discovery of the tetanus bacillus by Nicolaier in 1884 and its successful cultivation by Kitasato in 1889, the true cause of the disease, which previously had been involved in mystery, was established. Kitasato in his investigations also found that the organism is not present in the blood of animals dying of the affection, and accordingly he concluded that the fatal results were produced by an intoxication and not by the infection. In his subsequent work with this disease he, together with Von Behring, successfully worked out and published an immunizing method in 1890.

It has already been stated that in tetanus, unlike most of the other infectious diseases, the organism itself does not exert the destructive influence, only the toxins of the organisms being responsible for the serious results of the disease. The tetanus bacilli therefore produce a specific substance which has the toxic effect. Kitasato came to this conclusion in the course of his experiment when he succeeded in producing typical cases of tetanus in mice, guinea pigs, rabbits, and other animals with the filtrate obtained by filtering tetanus cultures through porcelain filters. At the same time he found that it required only a remarkably small quantity of the toxin to produce tetanus with a fatal termination in these animals

In the above-mentioned epoch-making work of Von Behring and Kitasato they attributed the established immunity to the effect of

the blood serum, which rendered harmless the toxic substances produced by the tetanus bacillus. From the following described experiments they obtained results demonstrating that the serum had an enormous power of destroying the poison:

Of a tetanus culture 10 days old which was freed from bacilli by filtration, 0.00005 c. c. was sufficient to kill a mouse in four to six days, and 0.0001 c. c. was sufficient to kill a mouse with certainty in two days. However, 5 c. c. of the serum from the tetanus-immune rabbits were mixed with 1 c. c. of this culture and the serum was allowed to act upon it for twenty-four hours. Four mice were each given 0.2 c. c. of the mixture, which contained 0.0033 c. c. of the culture, or more than 300 times the dose otherwise fatal for mice. All four of the mice remained well. The control mice, on the other hand, died in thirty-six hours after having received 0.0001 c. c. of the fluid. As a result of these and other experiments the authors drew the following conclusions:

1. The blood of tetanus-immune rabbits possesses the property of destroying the tetanus poison.

2. This property is destroyed by the extra vascular blood and the cell-free serum obtained from it.

3. This property is of such a stable nature that it is also effective in the bodies of other animals, so that we are in position to accomplish noteworthy therapeutic results by means of the transfer of blood serum.

4. The property of destroying the tetanus poison is absent in the blood of animals which have not been immunized against tetanus. If tetanus be given to susceptible animals the toxin may be demonstrated in the blood and other body fluids after the death of the animals.

Tizzoni and Cattain have also established that the blood of artificially immunized pigeons and dogs has a protective action on mice and rats against the tetanus toxin (1 to 2 drops of the dog serum made 0.5 c. c. of a toxic culture filtrate ineffective).

With these wonderful results as a foundation, the serum therapy of tetanus was established.

The practical application of this method was inaugurated after Schütz had found that horses and sheep can also be successfully immunized against tetanus and that they produce an active immunizing serum. Horses are now exclusively used for the production of tetanus antitoxin, as large quantities of blood may be drawn from an immunized horse, which constitutes a great advantage in the manufacturing of this product for the market.

The immunizing serum is prepared according to Von Behring's method in the following way: Of 200 c. c. of a virulent bouillon culture, of which 0.75 c. c. kills a rabbit in three to four days, 80 c. c. is mixed with sufficient trichlorid of iodin to make a 0.25 per cent solution; similarly, 60 c. c. is mixed with enough to make a 0.175 per cent solution, and 40 c. c. with sufficient to make a 0.125 per

cent solution, while the remaining 20 c. c. of the culture is left without any addition. The horse is then injected every eight days, commencing with 10 to 20 c. c. of the greatest dilution and continuing successively with the weaker attenuations of the culture. Finally the pure culture is injected, commencing with 0.5 c. c. and doubling the dose every five days, until no reaction to the culture is manifested and the blood shows the highest number of immunity units obtainable from that individual animal.

MODE OF ACTION OF TETANUS TOXIN.

The highly poisonous qualities of this toxin and the fact that the organism causing the disease acts only through the generated toxin induced a greater number of investigators to carry out various investigations in connection therewith. The tetanus bacillus, it will be remembered, does not enter the general organism, but remains at the point of entrance (wounds) and only exerts its destructive effect through the action of the toxins which it eliminates. From the place of infection the toxin is conveyed principally by the nerves to the central nervous system, and here is its point of attack. The central nervous system, and especially the spinal cord, is first affected by the toxin, and all functional manifestations of the intoxication, as well as the increased reflex irritability, such as the tonic spasms of the muscles, are the result of the poisoning of the nerve cells of the spinal cord. That the muscle itself is not directly influenced in its function by the tetanic poison is no longer doubted by pathologists.

The tetanus toxin is taken up by the peripheral nerve endings and is then conducted by the nerves to the medullary centers, where it combines with the motor nerve cells. The poisoning of these cells then produces the tonic muscular spasms as well as the reflex irritability (Meyer and Ransom, Bruschettini, Stinzing, Von Behring, and others).

The mode of action of the tetanus toxin is explained by Ehrlich's side-chain theory in the following way: Every toxin molecule consists of a nonpoisonous (haptophore) and a poisonous (toxaphore) atom group. On the other hand, the protoplasm of the motor nerve cells consists of a vital nucleus and of numerous side chains (receptors), of which some possess a special affinity toward the haptophore atom groups of the toxin molecules. If molecules of the tetanus poison enter the nerve cells, they become anchored to the corresponding receptors of the cell protoplasm by the haptophore atom group, and their toxophore group exerts a harmful effect on the vital nucleus of the cell. There follows either a total destruction of the cell protoplasm as a result of this combination or a stimulation of the cell protoplasm to defensive action which is manifested by the continual production and elimination of the receptors into the blood.

The presence of these free receptors in the blood of immunized animals explains the antitoxic immunity, since these receptors are enabled to combine with the haptophore atom of the toxin molecule before it has had an opportunity to attack the cell protoplasm.

The tetanus toxin, according to Ehrlich, contains at least two poisons, the tetano-lysin and the tetano-spasmin. He showed that on the relative proportions of these poisons depends whether the toxin has stronger tetanic properties and a weaker hemolytic action, or vice versa.

TOXICITY OF TETANUS TOXIN.

Testing the tetanus toxin for its virulence, Kitasato found that 0.0002 c. c. of the filtrate of a tetanus culture proved fatal to a mouse, while 0.002 c. c. killed a guinea pig weighing 560 grams. A rabbit, on the other hand, required relatively double the quantity of the mouse and almost seven times as much as the guinea pig, since it required 0.04 c. c. of the filtrate to kill a rabbit weighing 1,490 grams. Accordingly, of these three species of experimental animals the guinea pigs are the most susceptible to tetanus toxin, mice coming next and rabbits last. Of all animals the horse is probably the most susceptible to this toxin.

The remarkable poisoning qualities of tetanus toxin can be seen from the findings of Briszer and Cohn; 0.00000005 gram of their strongest tetanus toxin killed mice weighing 15 grams. Smaller doses, as 0.00000001 gram, caused tetanic symptoms in mice. The minimal lethal dose (MLD) of the standard tetanus toxin of the Hygienic Laboratory of the United States Public Health and Marine-Hospital Service is 0.000006 gram for a guinea pig weighing 350 grams.

STABILITY OF TETANUS TOXIN.

Kitasato was the first to make extensive tests on the stability of the tetanus toxin, and while other investigators have since undertaken similar work with it the results they have obtained merely confirmed the findings of Kitasato.

In experimenting upon the effect of physical influences on the filtrate, Kitasato found that tetanus toxin is quite susceptible to heat. Subjected to 65° C. and above, it is totally destroyed in five minutes, while it resists 60° for fifteen minutes, but twenty minutes' exposure to this temperature destroys it. The drying of the filtrate does not necessarily destroy its virulence. However, a great deal depends upon the method of drying, as for instance, if dried in the incubator it is entirely destroyed, while if dried in the exsiccator over sulphuric acid or in the air at room temperature it retains its virulence. Exposure of the filtrate to diffused light showed a gradual diminishing effect

upon its toxic action; however, it requires a long time before its virulence is entirely destroyed, as the filtrate which had been exposed for nine to ten weeks to the diffused light in the window still had toxic effects in large doses. On the other hand, the filtrate retains its virulence for a very long period if kept cold in a dark room, since it did not lose any of its virulence after three hundred days under such conditions. Exposure of the filtrate to direct sunlight proved that it is entirely destroyed only after fifteen to eighteen hours, while action of the filtrate. Dilutions of the filtrate with water or bouillon shorter exposure showed a corresponding diminution of the toxic does not influence the virulence of the tetanus poison.

Of the chemical influences on the filtrate Kitasato found that tetanus toxin is very susceptible to the action of acids (especially mineral acids) as well as alkalies.

Practically the same results were obtained by Rosenau and Anderson regarding the stability of the tetanus toxin, and as a result of their extensive investigation with the dried toxin they found that it not only retains its virulence when kept in a cold, dark place, but loses its toxicity quite slowly when exposed to light, heat, and other influences. One sealed tube sent from Washington to Manila by mail arrived there without appreciable loss of toxicity. The stability of the dried toxin is of great advantage in the work of standardization of the antitoxins, as it assures accurate work and also simplifies and expedites the tests.

THE STANDARDIZATION OF TETANUS ANTITOXIN.

EUROPEAN METHODS.

With the establishment of the principles of immunizing against tetanus by Von Behring and Kitasato, it became necessary to adopt a method by which the potency of the antitoxin could be accurately determined. In Germany the testing of the tetanus antitoxin is carried out in accordance with the Von Behring method, which provides that normal serum shall be a serum of which 0.1 c. c. renders ineffective 0.03 gram of normal toxin. This normal or test toxin is a dried toxin, 1 gram of which has a virulence capable of killing 10,000,000 mice each weighing 15 grams. The Italian method of standardizing tetanus antitoxin is based on the work of Tizzoni and Cattain. The toxin unit, according to this method, is the smallest amount of toxin which will kill a rabbit weighing 1 kilogram in from four to five days The Tizzoni antitoxin contains 80,000 immunity units in every cubic centimeter; in other words, this amount of antitoxin will neutralize 80,000 toxin units.

The French method of standardizing tetanus antitoxin is carried out by the subcutaneous inoculation of a series of guinea pigs with

quantities of serum equal to $\frac{1}{500000}$, $\frac{1}{1000000}$, $\frac{1}{1500000}$, etc., of their weight. Twenty hours later these test animals are given a single fatal dose of toxin and the immunizing power of the serum is then considered to be $\frac{1}{500000}$ or $\frac{1}{1000000}$, etc., dependent upon whether the animal receiving this proportional weight of serum has survived the toxin which proved fatal to the control guinea pig.

THE AMERICAN METHOD OF STANDARDIZATION.

The method of standardization which was carried out in connection with the testing of the veterinary antitoxins as reported in this paper followed the exact lines of the method known as the American method, which has been adopted officially under the biological product act of July 1, 1902.

This method, which is the result of several years' work on this subject in the Hygienic Laboratory, is a highly creditable achievement by Rosenau and Anderson,[a] as it not only simplifies the standardization of this valuable serum, but is also perfectly reliable in its accuracy. The method was also unanimously adopted by the Society of American Bacteriologists subsequent to the following report made by a special committee:

> That tetanus antitoxin be standardized by the tetanus toxin furnished by the Public Health and Marine-Hospital Service. The unit is ten times the least amount of serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of the standard toxin. The test dose is 100 minimal lethal doses of a precipitated toxin preserved under special conditions at the Hygienic Laboratory of the Public Health and Marine-Hospital Service. It was decided that the minimal immunizing dose for a case of possible infection through a wound should be 1,500 of such units. It was decided that after April 1 the new unit should be adopted by all producers of tetanus antitoxin.
>
> J. J. Kinyoun, *Chairman.*
> Herbert D. Pease,
> Joseph McFarland,
> Theobald Smith,
> E. M. Houghton,
> M. J. Rosenau,
> William H. Park, *Secretary.*

In this method the immunity unit for measuring the strength of tetanus antitoxin is fixed so that it shall be ten times the least quantity of antitoxin serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine-Hospital Service.

Thus it is required from the manufacturers of the "human" tetanus antitoxin to state the number of units their products contain, which

[a] Rosenau and Anderson. The standardization of tetanus antitoxin. Bulletin 43, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service. Washington, 1908.

not only insures serum of reliable strength, but also establishes a uniformity among the producers of tetanus antitoxin in America. On the other hand, the antitoxins destined for "veterinary" use are still under no control whatsoever; there is no uniformity in the method of standardization, and the potency of the product is absolutely left to the honesty of the manufacturer. This state of affairs of course does not assure the veterinarian of any uniformity of the product even of the same manufacturer, still less of different manufacturers. Only one of the different veterinary tetanus antitoxins on the market states the number of American units the immunizing or curative dose contains. The others fail to make any declaration, and in one case the number of units is given in the hundred thousands, whereas the human antitoxin of the same firm of course complies with the requirements of the law, stating definitely the number of American units that particular antitoxin contains. These conditions alone should suffice to point out the necessity for supervision of this veterinary product and the establishment of uniformity among the producers of veterinary antitoxin. It is very essential that the veterinarian should have some assurance of the strength of the antitoxin upon which his standing as a professional man may depend.

EXAMINATION OF COMMERCIAL VETERINARY ANTITOXINS.

The samples of tetanus antitoxin (veterinary) which were standardized in connection with this work were obtained from various drug stores in Chicago, New York, and Washington. They were kept at a temperature from 50° to 60° C. until the tests were made, care being taken not to expose them to any condition which might affect the potency of the antitoxin.

The toxin was obtained from the Hygienic Laboratory of the Public Health and Marine-Hospital Service through the courtesy of Surgeon-General Walter Wyman, and represented the dried standard toxin which is used in the standardization of tetanus antitoxin in the Hygienic Laboratory and furnished to the manufacturers of antitoxin. In the determination of the value of the antitoxin the L+ dose is the test dose of the toxin. The L+ dose is the smallest quantity of tetanus toxin that will neutralize one-tenth of an immunity unit, plus a quantity of toxin sufficient to kill an animal in just four days. The L+ dose of the toxin which was used in these tests contained 100 minimal lethal doses (MLD) for a 350-gram guinea pig. The toxin was kept in a dark, cold place and during the course of these tests its virulence was controlled by repeated tests with an antitoxin of known value.

The guinea pigs used in connection with this standardization were carefully selected, being vigorously healthy animals of from 350 to 370 grams in weight. The glassware employed in the work was

selected according to the recommendation of Rosenau, and was not used for any other purpose. The mixing cylinders and mixing flasks were the kind designed by Rosenau. Ehrlich's delivery pipettes graduated into hundredths, as well as special delivery and capacity pipettes, were used. The syringes employed were a modification of the old Koch syringe, in which the barrel tapers gradually to the needle, so that the last drop runs out readily.

The necessary measures against bacterial contamination were taken during the execution of the work. All the glassware was first rendered chemically clean and then sterilized for one hour at 120° C. All the various steps in the procedure were followed carefully in order that the results would be accurate and reliable. The method of the standardization was executed in every particular as described by Rosenau and Anderson in Bulletin 43 of the Hygienic Laboratory.

The number of immunity units contained in a cubic centimeter of serum is determined by the quantity of antitoxin which saved the life of a guinea pig for ninety-six hours against the official test dose of the toxin. This quantity of antitoxin represents one-tenth of an immunity unit. For instance, if a guinea pig receiving 0.0015 c. c. of the antitoxin with the official test dose of toxin is saved for ninety-six hours, this quantity of antitoxin contains one-tenth of an immunity unit. Accordingly, the following equation is represented: $0.0015 : 0.1 :: 1 : x$, which indicates that 1 cubic centimeter of the serum contains 66 units.

In the following tables are given the results of the standardization obtained from the tests of the various samples of veterinary tetanus antitoxin examined in this laboratory:

TABLE 1.—*H. K. Mulford Company's Tetanus Antitoxin Serum for veterinary use. Immunizing dose, labeled to contain 500,000 units. To be exchanged after May 1, 1910. Laboratory No. 2960. Syringe contained 7.6 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	c. c.			
1.....	0.0006	0.0010	1 day, 20 hours.		
2.....	.0006	.0011	1 day, 26 hours.		
3.....	.0006	.0012	3 days, 19 hours.		
4.....	.0006	.0013	5 days, 22 hours	81	615
5.....	.0006	.0014	Symptoms.		
6.....	.0006	.0015	No symptoms.		
7.....	.0006	.0020	Do.		
8.....	.0006	.0025	Do.		
9.....	.0006	.0030	Do.		

TABLE 2.—*Parke, Davis & Co.'s Antitetanic Serum (veterinary). To be exchanged after November 24, 1910. Unit value not stated. Syringe contained 10 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	c. c.			
1	0.0006	0.0006	1 day, 12 hours.		
2	.0006	.0007	3 days, 7 hours.		
3	.0006	.0008	4 days, 12 hours	125	1,250
4	.0006	.0009	Symptoms.		
5	.0006	.0010	Slight symptoms.		
6	.0006	.0015	No symptoms.		
7	.0006	.0020	Do.		
8	.0006	.0025	Do.		
9	.0006	.0030	Do.		

TABLE 3.—*H. K. Mulford Company's Tetanus Antitoxin Serum for veterinary use. Immunizing dose, labeled to contain 500,000 units. To be exchanged May 15, 1910. Laboratory No. 2960. Syringe contained 7.5 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	c. c.			
1	0.0006	0.0010	2 days, 12 hours.		
2	.0006	.0013	4 days, 7 hours	81	607
3	.0006	.0016	6 days, 8 hours.		
4	.0006	.0019	Slight symptoms.		
5	.0006	.0022	No symptoms.		

TABLE 4.—*Pasteur Laboratories, Paris, France, Antitetanic Serum for veterinary use. Unit value not stated. Bottle contained 10 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in bottle.
	c. c.	c. c.			
1	0.0006	0.0010	4 days, 2 hours	100	1,000
2	.0006	.0013	Slight symptoms.		
3	.0006	.0016	No symptoms.		
4	.0006	.0019	Do.		
5	.0006	.0022	Do.		

TABLE 5.—*Lederle Antitoxin Laboratories, Tetanus Antitoxin (veterinary). Immunizing dose, labeled to contain 1,500 units. Laboratory No. 19A. To be exchanged June 21, 1910. Syringe contained 10.4 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	*c. c.*			
1	0.0006	0.00035	1 day, 18 hours.		
2	.0006	.00050	1 day, 15 hours.		
3	.0006	.00065	4 days, 3 hours	154	1,601
4	.0006	.00080	Symptoms.		
5	.0006	.00085	No symptoms.		

TABLE 6.—*Parke, Davis & Co's Antitetanic Serum (veterinary). To be exchanged after December 8, 1910. Unit value not stated. Syringe contained 10 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	*c. c.*			
1	0.0006	0.0005	3 days, 1 hour.		
2	.0006	.0008	5 days, 20 hours	125	1,250
3	.0006	.0011	No symptoms.		
4	.0006	.0014	Do.		
5	.0006	.0017	Do.		

TABLE 7.—*Parke, Davis & Co's Antitetanic Serum (veterinary). To be exchanged after December 8, 1910. Unit value not stated. Syringe contained 10 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	*c. c.*			
1	0.0006	0.0005	2 days, 21 hours.		
2	.6006	.0008	Symptoms	125	1,250
3	.0006	.0011	Slight symptoms.		
4	.0006	.0014	No symptoms.		
5	.0006	.0017			

TABLE 8.—*Parke, Davis & Co's Antitetanic Serum (veterinary). To be exchanged after January 14, 1911. Unit value not stated. Syringe contained 10 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	c. c.			
1.....	0.0006	0.0010	4 days, 5 hours.....	100	1,000
2.....	.0006	.0014	Slight symptoms.		
3.....	.0006	.0018	No symptoms.		
4.....	.0006	.0022	Do.		
5.....	.0006	.0026	Do.		

TABLE 9.—*H. K. Mulford Company's Tetanus Antitoxin Serum for veterinary use. Immunizing dose, labeled to contain 500,000 units. To be exchanged after May 15, 1910. Laboratory No. 2971. Syringe contained 8 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	c. c.			
1.....	0.0006	0.0008	2 days, 1 hour.		
2.....	.0006	.0011	2 days, 18 hours.		
3.....	.0006	.0014	2 days, 23 hours.		
4.....	.0006	.0017	6 days, 4 hours.....	59	472
5.....	.0006	.0020	Slight symptoms.		

TABLE 10.—*Pasteur Laboratories, Paris, France, Antitetanic Serum for veterinary use. Unit value not stated. Bottle contained 10 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in bottle.
	c. c.	c. c.			
1.....	0.0006	0.0006	2 days, 20 hours.		
2.....	.0006	.0009	6 days, 7 hours.....	111	1,110
3.....	.0006	.0012	Symptoms.		
4.....	.0006	.0015	No symptoms.		
5.....	.0006	.0018	Do.		

TABLE 11.—*Pasteur Laboratories, Paris, France, Antitetanic Serum for veterinary use. Unit value not stated. Bottle contained 10 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in bottle.
	c. c.	c. c.			
1.....	0.0006	0.00050	3 days, 6 hours.		
2.....	.0006	.00065	4 days, 18 hours	153	1,530
3.....	.0006	.00080	No symptoms.		
4.....	.0006	.00095	Do.		
5.....	.0006	.00110	Do.		

TABLE 12.—*H. K. Mulford Company's Tetanus Antitoxin Serum for veterinary use. Immunizing dose, labeled to contain 500,000 units. To be exchanged after May 1, 1910. Laboratory No. 2,960. Syringe contained 7.5 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	c. c.			
1.....	0.0006	0.0007	1 day 14 hours.		
2.....	.0006	.0010	3 days 7 hours.		
3.....	.0006	.0013	Symptoms	81	607
4.....	.0006	.0016	No symptoms.		
5.....	.0006	.0019	Do.		

TABLE 13.—*Lederle Antitoxin Laboratories, Tetanus Antitoxin (veterinary). Immunizing dose, labeled to contain 1,500 units. Laboratory No. 19A. To be exchanged June 21, 1910. Syringe contained 8.5 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in syringe.
	c. c.	c. c.			
1.....	0.0006	0.00024	1 day 16 hours.		
2.....	.0006	.0003333	2 days 22 hours.		
3.....	.0006	.00043	Symptoms	232	1,972
4.....	.0006	.00053	No symptoms.		
5.....	.0006	.00060	Do.		

TABLE 14.—*Pasteur Laboratories, Paris, France. Antitetanic Serum for veterinary use. Unit value not stated. Bottle contained 10 c. c.*

No. of guinea pig.	Subcutaneous injection.		Time of death or effect of injection.	Antitoxin units.	
	Toxin.	Antitoxin.		Number per cubic centimeter.	Total number in bottle.
	c. c.	c. c.			
1	0.0006	0.00035	1 day 14 hours.		
2	.0006	.00050	2 days 20 hours.		
3	.0006	.00065	4 days 5 hours	153	1,530
4	.0006	.00080	4 days 16 hours.		
5	.0006	.00095	Slight symptoms.		

The results of these tests clearly demonstrate the variations in the potency of veterinary tetanus antitoxins at present on the market. While the preparations of the individual manufacturers do not show such marked differences in strength, yet they do not uniformly contain a sufficient number of units.

In accordance with the law of 1902, the manufacturers of human antitoxins are required to state on the labels of the packages the number of units that the particular antitoxin possesses. Should it be found on investigation that the antitoxin does not come to within 10 per cent of the strength stated on the labels, the manufacturer is immediately required to recall from the market all that particular antitoxin. Thus, if a physician intends to use the antitoxin either for immunizing purposes or as a curative agent, he is accurately guided in the dose by the statement on the label. It does not matter who the manufacturer of the antitoxin is. This latter fact is in itself also of great importance, as frequently the drug stores carry antitoxins of only one or two manufacturers.

On the other hand, the veterinarian has not always the good fortune of knowing the number of units that an antitoxin which he purchases contains. Only one of the manufacturers states on the label the number of American units contained in his veterinary antitoxin. One other manufacturer still uses for his veterinary antitoxin a standardization—other than American—by which he can label his product in the hundred thousands, yet his antitoxin for the human gives the units in the American standard. Why should two different standards be maintained, one for the human and the other for veterinary antitoxin?

The veterinary tetanus antitoxins are marketed in immunizing doses and curative doses. The immunizing dose is supposed to contain 1,500 American units. The volumetric quantity of this dose was found to be accurately 10 c. c. in the Parke, Davis & Co. and the Pasteur product, while the Lederle serum contained from 8.5 c. c. to 10.4 c. c. and the Mulford syringe from 7.5 to 8 c. c. Now, should

it be desired to administer to a horse an immunizing dose of the antitoxin it can readily be seen from the results obtained in these tests, as indicated by the tables, that while the immunizing dose of some of the serums contains the desired 1,500 units, others, on the other hand, possess less than one-third of that strength. For instance, according to the test shown by Table 9, the syringe contained 8 c. c. of serum with 59 units per cubic centimeter; thus the immunizing dose in this case represents only 472 units, and of course the curative dose is correspondingly low. This alone is sufficient to indicate the urgent necessity for some uniformity in standardizing the veterinary antitoxins, and also for Federal legislation by which they could be subjected to a periodical control with reference to their potency.

Under the present conditions there is the constant uncertainty regarding the strength of the serum, as the veterinarian has no assurance whatever of its potency, and is solely dependent on the reliability of the manufacturer.

CONCLUSIONS.

1. The veterinary tetanus antitoxins prepared by the different manufacturers have not a uniform potency, and the variation amounts in some instances to about two-thirds less than the strength which it should possess.

2. In order to insure a uniform strength, the manufacturers of veterinary tetanus antitoxins should be required to use the American standard, and to state on the label the number of American units the dose contains, as is required for human tetanus antitoxin.

3. The immunizing dose for a horse should contain at least 1,500 immunity units of the standard established by the United States Public Health and Marine-Hospital Service.

4. It is seen that the veterinary tetanus antitoxins vary extravagantly in the unit strength, and some are comparatively weak in antitoxic potency, which shows the necessity for the same supervision by the United States Department of Agriculture over biological products used in veterinary medicine as is now exercised by the United States Public Health and Marine-Hospital Service over similar products used in human medicine.

5. The request for such supervision should have the indorsement of the veterinarians and live-stock interests of this country.

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